Listing of Claims:

1. (Currently amended) An in vitro method of activating protein kinase B comprising the steps of:

(a) washing and cooling insulin responsive cells in ice cold buffer,

(b) incubating said insulin responsive cells from (a) in the presence of insulin,

(c) obtaining from said insulin-responsive cells from (b) a membrane fraction ~~fraction,~~ which comprises (i) an insulin receptor, (ii) an IRS-1("insulin receptor substrate -1"), (iii) an IRS-2 ("insulin receptor substrate -2"), (iv) a p85 subunit of PI-3 Kinase ("phosphatidyl-inositol 3-kinase") and (iv) PDK2 ("phosphoinositide-dependent kinase 2") activity, and a cytoplasmic fraction ~~fraction,~~ which comprises (i) PDK1 ("phosphoinositide-dependent kinase 1") activity, (ii) an IRS-1("insulin receptor substrate -1"), (iii) an IRS-2 ("insulin receptor substrate -2"), (iv) a p85 subunit of PI-3 Kinase ("phosphatidyl-inositol 3-kinase") and ~~which comprises~~ (v) a protein kinase B,

~~(b)~~ (d) preparing an assay mixture comprising the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride,

~~(c) optionally adding a phosphatidylinositol phosphate compound to the assay mixture,~~ and

~~(d)~~ (e) incubating said assay mixture at about 37°C for up to about 15 minutes;

~~Wherein~~ wherein following step (e) said insulin receptor is autophosphorylated, said IRS-1 and said IRS-2 are phosphorylated, said p85 subunit of PI 3-kinase is bound to said IRS-1 and to said IRS-2, a PI 3-kinase is activated, said PDK1 is activated, said PDK2 is activated, and said protein kinase B is activated in the assay mixture by virtue of having a threonine residue phosphorylated and a serine residue phosphorylated such that the activated protein kinase B is capable of phosphorylating a GSK3 ("glucose synthase kinase-3"), thereby activating protein kinase B in vitro.

2. (Canceled)

3. (Currently amended) The method of Claim 1 ~~claim 2~~ wherein the membrane fraction is a plasma membrane fraction.

4. (Original) The method of Claim 1 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.

5. (Previously presented) The method of Claim 1 further comprising the step of combining PIP3 ("phosphatidylinositol 3,4,5-triphosphate") or PI(3,4)P2 ("phosphatidylinositol 3,4-biphosphate") with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

6. (Original) The method of Claim 5 further comprising the step of combining PIP3 with the membrane fraction, the cytoplasmic fraction and ATP in a buffer comprising less than 145 mM chloride.

7. (Original) The method of Claim 1 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

8. (Original) The method of Claim 1 wherein the insulin-responsive cell is an adipocyte.

9. (Currently amended) An in vitro method of activating protein kinase B comprising:

(a) obtaining from an insulin-responsive cell a plasma membrane fraction ~~fraction,~~ which comprises a PDK2 ("phosphoinositide-dependent kinase 2") activity and a cytoplasmic fraction ~~fraction,~~ which comprises a protein kinase B and a PDK1 ("phosphoinositide-dependent kinase 1") activity,

(b) treating said plasma membrane fraction with a solution comprising at least 145 mM chloride, thereby obtaining a salt-extracted plasma membrane fraction and an aqueous fraction,

(c) desalting the aqueous fraction thereby producing a desalted aqueous fraction comprising less than 145 mM chloride and said PDK2 ("phosphoinositide-dependent kinase 2") activity,

(d) preparing an assay mixture comprising the salt-extracted plasma membrane fraction, the cytoplasmic fraction, the desalted aqueous fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride, wherein

(e) the protein kinase B is activated in the assay mixture by virtue of having a threonine residue phosphorylated and a serine residue phosphorylated, such that

(d) the activated protein kinase B is capable of phosphorylating a GSK3.

10. (Original) The method of Claim 9 wherein the serine residue is at a position corresponding to amino acid 473 of SEQ ID NO:1 and the threonine residue is at a position corresponding to amino acid 308 of SEQ ID NO:1.

11. (Original) The method of Claim 9 wherein the insulin-responsive cell is a muscle cell, a liver cell, an adipocyte or an islet cell.

12. (Original) The method of Claim 9 wherein the insulin-responsive cell is an adipocyte.

13. (Original) The method of Claim 9 wherein the insulin-responsive cell is treated with insulin.

14. (Original) The method of Claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3 or PI(3,4)P2.

15. (Original) The method of Claim 9 wherein the phosphatidylinositol phosphate molecule is a PIP3.

16-29. (Canceled)

30. (New) The method of Claim 1 wherein said insulin responsive cells are incubated in ice cold buffer in step (b).

31. (New) The method of Claim 1 further comprising the step of adding a phosphatidylinositol phosphate compound to the assay mixture in step (d).

32. (New) The method of Claim 31 wherein said insulin responsive cells are incubated in the absence of insulin in step (b).